

Analysis of environmental drivers influencing interspecific variations and associations among bloom-forming cyanobacteria in large, shallow eutrophic lakes

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ABSTRACT

Non-diazotrophic *Microcystis* and filamentous N_2 -fixing *Aphanizomenon* and *Dolichospermum* (formerly *Anabaena*) co-occur or successively dominate freshwaters globally. Previous studies indicate that dual nitrogen (N) and phosphorus (P) reduction is needed to control cyanobacterial blooms; however, N limitation may cause replacement of non- N_2 -fixing by N_2 -fixing taxa. To evaluate potentially counterproductive scenarios, the effects of temperature, nutrients, and zooplankton on the spatio-temporal variations of cyanobacteria were investigated in three large, shallow eutrophic lakes in China. The results illustrate that the community composition of cyanobacteria is primarily driven by physical factors and the zooplankton community, and their interactions. Niche differentiation between *Microcystis* and two N_2 -fixing taxa in Lake Taihu and Lake Chaohu was observed, whereas small temperature fluctuations in Lake Dianchi supported co-dominance. Through structural equation modelling, predictor variables were aggregated into 'composites' representing their combined effects on species-specific biomass. The model results showed that *Microcystis* biomass was affected by water temperature and P concentrations across the studied lakes. The biomass of two filamentous taxa, by contrast, exhibited lake-specific responses. Understanding of driving forces of the succession and competition among bloom-forming cyanobacteria will help to guide lake restoration in the context of climate warming and N:P stoichiometry imbalances.

1. Introduction

Harmful algal blooms caused by cyanobacteria are a notorious symptom of eutrophication and have detrimental impacts on recreation, ecosystem integrity, and human and animal health (Downing et al., 2001; Smith and Schindler, 2009). Owing to the rising mean global temperatures and anthropogenic eutrophication, climate change catalyzes the proliferation and expansion of cyanobacteria blooms (Paerl and Huisman, 2009; Paerl and Paul, 2012; Rigosi et al., 2015). Many cyanobacteria taxa, unlike eukaryotic phytoplankton, have eco-physiological traits that enable them to have a competitive advantage under warmer and nutrient-rich conditions. For example, some genera are efficient users of molecular carbon dioxide and are more likely to accumulate surface scums; and some taxa are able to fix nitrogen from the atmosphere and are highly competitive under a low phosphorus

condition (Carey et al., 2012; O'neil et al., 2012). Cyanobacteria represent a diverse and heterogeneous assemblage and not all taxa can form blooms. It is important to examine the external drivers for bloom-forming taxa, because multiple strains vary in physiological responses to environmental conditions (Sebastián et al., 2005; Wells et al., 2015).

Lakes experiencing frequent cyanobacterial blooms undergo succession of dominant species (Soares et al., 2009; Tsukada et al., 2006). Previous studies have shown that the most common bloom-forming cyanobacteria in productive temperate lakes are *Dolichospermum* (*Anabaena*), *Aphanizomenon*, and *Microcystis* (Paerl and Otten, 2015). Seasonal succession may be summarized as follows: N_2 -fixing *Aphanizomenon* occurs earlier in early spring, followed by the dominance of non-diazotrophic *Microcystis* during peak summer, then another N_2 -fixer, *Dolichospermum*, gradually dominates in the fall and winter (Moisanter et al., 2008; Wu et al., 2016). Spatially, different dominant

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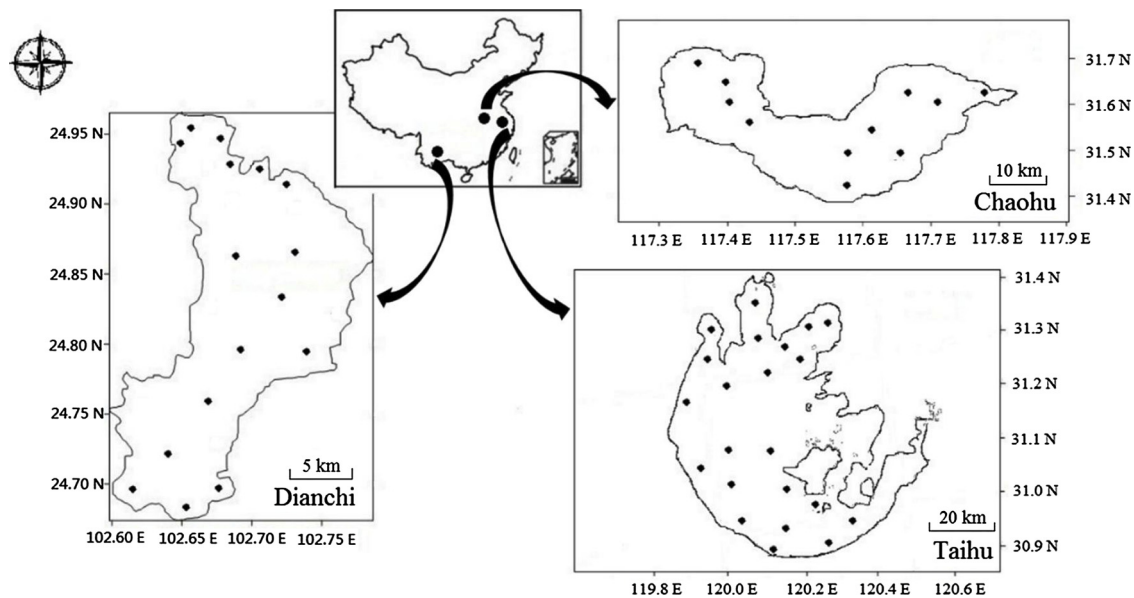


Fig. 1. Map of three large, shallow lakes in China including Lake Taihu, Lake Chaohu and Lake Dianchi. Water samples from different sites were routinely collected at stations indicated by dots in the lakes.

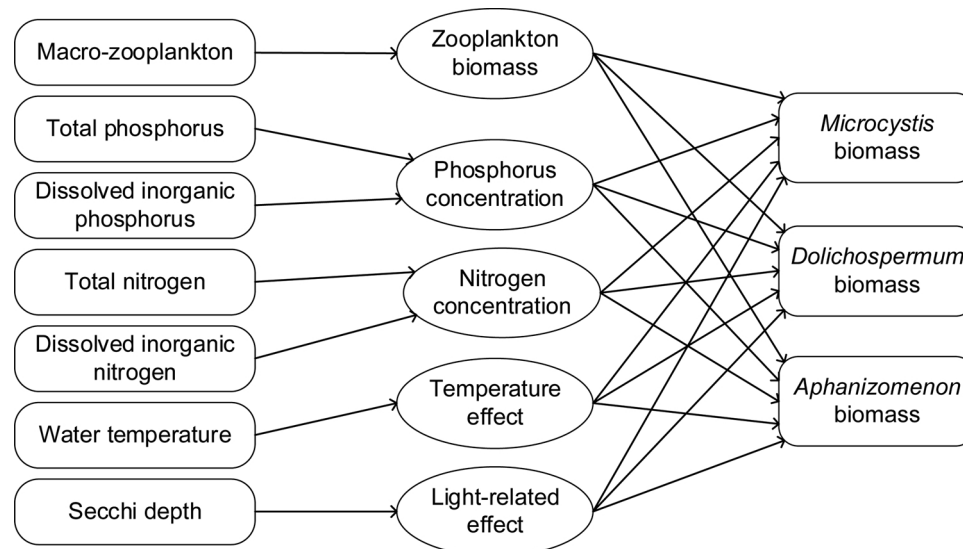


Fig. 2. The *a priori* structural equation model used to assess the biomass of three bloom-forming taxa. Exogenous environmental predictor variables (boxes on left) are combined into composite variables (circles) and used to predict the variation in the response variables (boxes on right).

species can co-exist in the same water body, or they dominate in different regions of a lake (Zhang et al., 2016). Factors causing the dominance or bloom of one or the other group are often difficult to determine, because the relative success of a cyanobacterial species is a result of complex and synergistic environmental factors rather than single dominant variables (Dokulil and Teubner, 2000; Hyenstrand et al., 1998).

There is strong evidence that the relative importance of temperature and nutrients to promote cyanobacteria is taxon dependent (Rigosi et al., 2014). Increasing dominance and geographic expansion of non- N_2 fixing *Microcystis* blooms have been observed in large lake systems (e.g., Lake Erie and Okeechobee, USA; Lake Tjeukemeer and Volkerak, Dutch; Lake Taihu, Chaohu and Dianchi, China), indicating N-enriched conditions (Donald et al., 2011; Paerl et al., 2014). Conversely, *Microcystis* blooms can selectively drive P release (but not N) from aerobic sediments by elevating pH, indicating their capability to decrease water column N:P ratios in shallow eutrophic ecosystems (Jensen and

Andersen, 1992; Yan et al., 2016). In a sense, P has longer residence time than N, leading to tendency of N-limited and N&P co-limited phytoplankton growth in many freshwater ecosystems (Harpole et al., 2011; Xu et al., 2010). The possibility exists that nitrogen reduction may cause replacement of filamentous non- N_2 fixing with N_2 -fixing taxa (Miller et al., 2013; Schindler et al., 2008). Filamentous taxa are the most common producers of taste-and-odor compounds (geosmin and 2-methylisoborneol) in drinking water supplies, thus presenting greater nuisance than *Microcystis* blooms.

Owing to the complex interactions of many environmental factors, cyanobacteria and zooplankton in different lakes are expected to respond differently to future climate warming and eutrophication (Tarau et al., 2012). Nonetheless, evidence from large-scale field data considering the dynamics of interspecific variations among cyanobacterial assemblages has been scarce (Wagner and Adrian, 2009). In this study, field observations were made from three largely bloom-dominated lakes, and statistical models were used in order to address

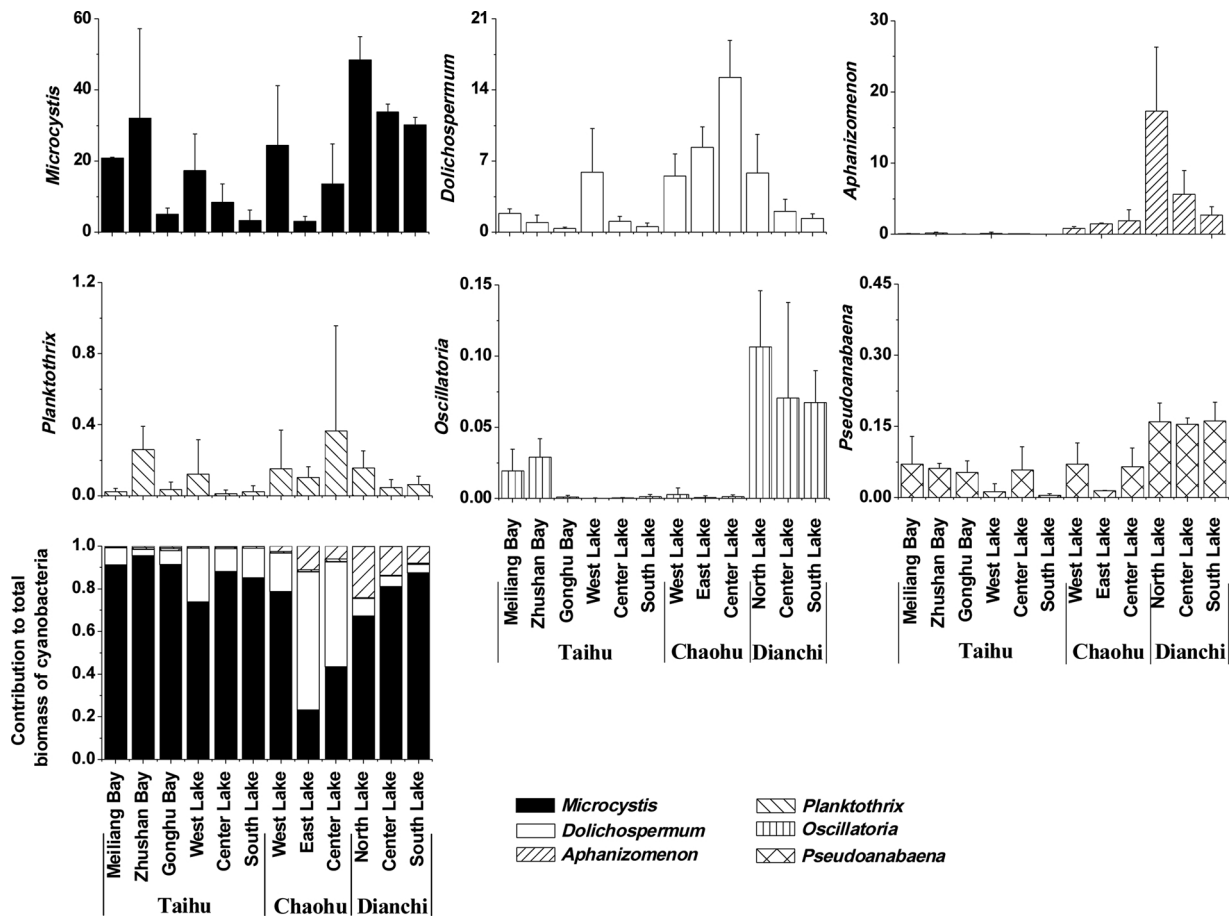


Fig. 3. Average values of wet biomass (mg/L) of the principal taxonomic cyanobacterial groups in different regions belongs to three lakes computed for the period 2008–2010. The vertical bars indicate the standard errors of the means.

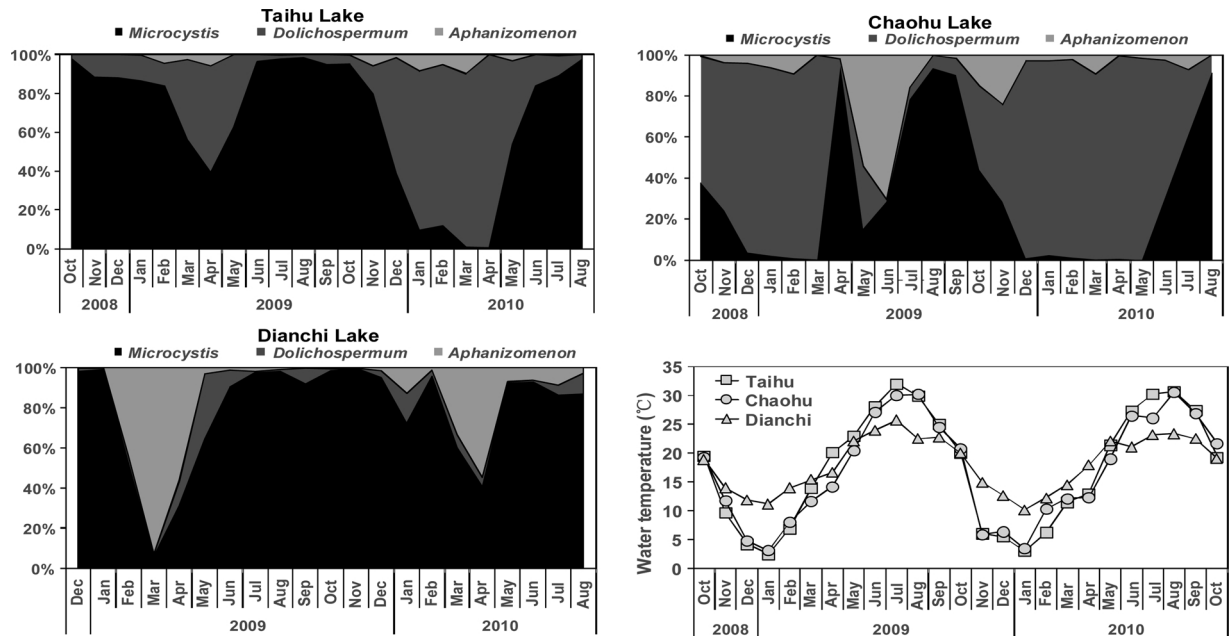


Fig. 4. Seasonal variations of proportion of three cyanobacteria species in Lake Taihu, Lake Chaohu, and Lake Dianchi. The lower right corner of the figure showed the monthly average temperature in the three studied lakes.

following objectives: (i) to compare spatial-temporal variations and environmental drivers for the cyanobacterial community based on lake-specific and across-lake datasets; (ii) to explore potential niche

differentiation under a range of possible temperature and nutrient windows; and (iii) to quantify the interactions of environmental and biological factors in the competitive relationship among bloom-forming

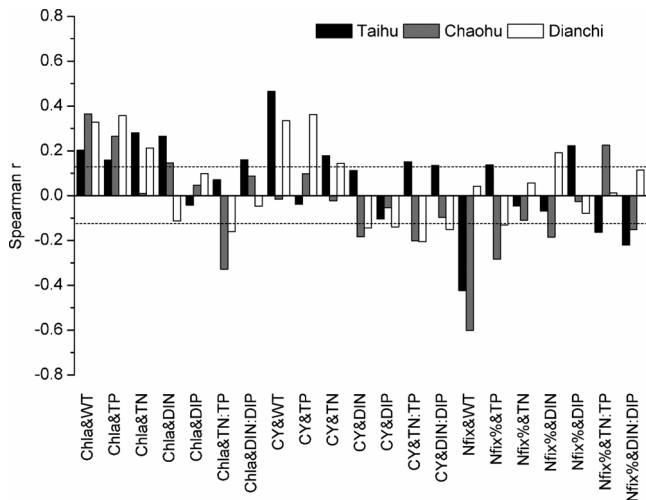


Fig. 5. Spearman correlations between Chla, total cyanobacteria biomass (CY) and N₂-fixing cyanobacteria in total cyanobacteria biomass (Nfix%), with water temperature (WT), total nitrogen (TN), dissolved inorganic nitrogen (DIN), total phosphorus (TP), dissolved inorganic phosphorus (DIP), the TN:TP and DIN:DIP ratios in three lakes. All $r > 0.12$ and $r < -0.12$ are significant at the $P < 0.01$ level.

species.

2. Materials and methods

2.1. Study area

The field samples were obtained monthly in 2008, 2009, and 2010 from Lake Taihu (TH), Lake Chaohu (CH), and Lake Dianchi (DC) in China (Fig. 1).

Lake Taihu, located at the center of the Yangtze River Delta in East China (30°56′31″N, 119°55′120″E), is the third largest freshwater lake in China. It covers an area of 2338 km² and a catchment area of 36,500 km². It has a water retention time of 284 days, with a mean depth of 1.9 m and a maximum depth of 2.6 m. Lake Taihu, mostly in Meiliang Bay (MLB), Zhushan Bay (ZSB), West Taihu (WTH), and Center Taihu (MTH), has suffered from *Microcystis* blooms; while Gonghu Bay (GHB) and South Taihu (STH) rarely experiences cyanobacterial blooms (Otten et al., 2012).

Lake Chaohu is the fifth largest freshwater lake in China. It is located in central Anhui Province (30°25′31″N, 117°17′117″E). It has a volume of 32.3×10^8 m³ in the rainy season with a residence time of 150 days, but has a volume of 17.2×10^8 m³ in the dry season with a residence time of 210 days. It covers a surface area of approximately 770 km², and a catchment area of about 9200 km², with a mean depth of 3.1 m and a maximum depth of 7.0 m. Lake Chaohu, mostly West

Chaohu (WCH), Center Chaohu (CCH) and East Chaohu (ECH), has suffered from the seasonal *Microcystis* and *Dolichospermum* blooms at different locations (Zhang et al., 2016).

Lake Dianchi, located in the southern part of Kunming City (24°29′–25°28′N, 102°29′–103°01′E), is the largest lake in Yunnan Plateau (Southwest of China). It has a surface area of 306 km², and a watershed area of 2920 km². Lake Dianchi is located at altitude 1887 m. The lake body has a mean depth of 4.7 m and a maximum depth of 10.9 m, with a residence time of approximately 3.5 years. Lake Dianchi, consisting of North Dianchi (NDC), Center Dianchi (CDC) and South Dianchi (SDC), has experienced spring-summer succession between *Aphanizomenon* and *Microcystis* blooms during 2009–2012 (Wu et al., 2016).

2.2. Sampling and laboratory methods

During each transect, water samples were collected at 0.5 m depth of surface layer. One liter of water sample was collected with a poly-methyl methacrylate sampler and preserved with acid Lugol's iodine solution (1% final conc.) for the identification of phytoplankton assemblages. Quantitative samples for protozoans and rotifers were prepared and preserved using the procedures used for the phytoplankton. Quantitative samples (20 L) for copepods and cladocerans were filtered through a 69 µm net, back-washed into a bottle with filtered lake water, and preserved in 4% formalin solution. Half of the water sample was brought back to the laboratory and filtered onto GF/C glass microfiber filters (1.2 µm, Whatman) for chlorophyll *a* (Chl *a*) by spectrophotometry after extraction in 90% hot ethanol (Pápišta et al., 2002). An additional one liter of water was collected and stored frozen at –20 °C in glass bottles until analysis for nutrient concentrations.

Water quality parameters, including surface water temperature (WT), conductivity, dissolved oxygen (DO), and pH were determined by multiprobe sonde (YSI 556MPS, USA) at 0.5 m depth of surface layer. DO and pH sensors were calibrated before deployment. Secchi depth (SD) representing transparency was measured with 10 cm diameter black and white disk. Wind speed (WS) was obtained from a meteorology station of the China Meteorological Administration. Total nitrogen (TN), dissolved inorganic nitrogen (DIN), total phosphorus (TP), and dissolved inorganic phosphorus (DIP) were measured according to previous descriptions (Wu et al., 2016).

The phytoplankton samples were concentrated via sedimentation for 48 h. The supernatant water was siphoned off, while a 30 mL volume of the remainder was kept for cell counting. Phytoplankton species were identified according to commonly used phytoplankton monographs and counted three times with a Sedgwick-Rafter counting chamber under Olympus CX31 optical microscope. Phytoplankton biomass was calculated according to abundance. The details of these steps are available in the study of Hu et al. (2016).

The samples for protozoa analysis used a volume of 0.1 mL in with 20 mm × 20 mm settling chambers. Samples for rotifer, copepod, and

Table 1

The average water temperature, total phosphorus, total nitrogen weighted by the biomass of three taxa and proportion of N₂-fixing taxa.

		<i>Microcystis</i> biomass	<i>Dolichospermum</i> biomass	<i>Aphanizomenon</i> biomass	Proportion of N ₂ -fixing taxa %
WT (°C)	All lakes	20.7	16.7	18.0	13.5
	Taihu	23.1	19.7	19.8	12.6
	Chaohu	22.9	13.1	16.7	13.1
	Dianchi	18.9	19.6	18.4	17.3
TP (mg/L)	All lakes	0.28	0.23	0.24	0.16
	Taihu	0.23	0.21	0.21	0.17
	Chaohu	0.35	0.18	0.18	0.12
	Dianchi	0.28	0.32	0.26	0.24
TN (mg/L)	All lakes	3.31	3.13	3.05	2.80
	Taihu	3.81	3.75	4.12	3.15
	Chaohu	3.38	2.59	2.76	2.42
	Dianchi	3.02	3.41	3.10	2.85

Table 2
The average of water quality parameters during the period from October 2008 to October 2010 on basis of monthly sampling.

Lake	Region (sample site)	avg.TN (mg/L)	avg.TP (mg/L)	avg.DIN (mg/L)	avg.DIP (mg/L)	ratio TN:TP	Ratio DIN:DIP	avg. Chl <i>a</i> (μg/L)	avg. SD (cm)	N ₂ -fixing Cyano%
Taihu	Meiliang Bay (n = 2)	3.97 ± 0.46	0.16 ± 0.01	1.13 ± 0.18	0.03 ± 0.00	30:1	73:1	60.66 ± 5.62	35 ± 2	8.3
	Zhushan Bay (n = 2)	4.65 ± 0.40	0.29 ± 0.09	1.66 ± 0.17	0.05 ± 0.00	25:1	107:1	48.12 ± 16.78	41 ± 3	3.4
	Gonghu Bay (n = 2)	3.43 ± 0.07	0.11 ± 0.00	0.86 ± 0.08	0.03 ± 0.00	35:1	55:1	16.15 ± 2.23	54 ± 3	6.9
	West Taihu (n = 4)	3.52 ± 1.06	0.22 ± 0.08	1.26 ± 0.34	0.04 ± 0.02	23:1	67:1	33.16 ± 6.83	25 ± 4	25.6
	Center Taihu (n = 5)	3.44 ± 0.39	0.16 ± 0.02	1.22 ± 0.22	0.04 ± 0.00	26:1	63:1	23.62 ± 1.75	31 ± 6	11.2
Chaohu	South Taihu (n = 7)	2.37 ± 0.27	0.15 ± 0.01	0.98 ± 0.12	0.03 ± 0.01	23:1	69:1	10.78 ± 3.02	31 ± 7	14.0
	West Chaohu (n = 4)	2.78 ± 0.16	0.22 ± 0.02	1.18 ± 0.25	0.04 ± 0.01	17:1	64:1	64.66 ± 12.60	30 ± 2	20.4
	East Chaohu (n = 3)	2.22 ± 0.21	0.10 ± 0.01	0.63 ± 0.02	0.02 ± 0.00	28:1	45:1	25.41 ± 0.81	47 ± 2	75.8
Dainchi	Center Chaohu (n = 4)	2.46 ± 0.31	0.15 ± 0.04	0.77 ± 0.15	0.03 ± 0.00	23:1	50:1	47.83 ± 12.32	39 ± 5	55.1
	North Dainchi (n = 6)	3.43 ± 0.79	0.31 ± 0.07	0.90 ± 0.34	0.05 ± 0.03	12:1	38:1	128.21 ± 35.32	24 ± 3	32.1
	Center Dainchi (n = 5)	2.25 ± 0.10	0.20 ± 0.01	0.48 ± 0.04	0.03 ± 0.00	16:1	25:1	86.92 ± 4.63	26 ± 1	18.3
	South Dainchi (n = 5)	2.33 ± 0.14	0.18 ± 0.00	0.40 ± 0.03	0.02 ± 0.01	17:1	38:1	73.67 ± 4.24	28 ± 2	11.6

cladoceran analysis used a volume of 1.0 mL with 50 mm × 20 mm × 1 mm settling chambers. Zooplankton was identified and enumerated according to commonly used monographs. Zooplankton biomass was calculated as wet weight per individual × abundance. Macro-zooplankton biomass was defined as the sum of the biomass of cladocerans and copepods.

2.3. Data analysis

To ensure that the residuals from the different statistical analyses were normally distributed and homogeneous, the biomass of three taxa (*Microcystis*, *Dolichospermum*, *Aphanizomenon*) was log transformed ($X_i + 1$) and is abbreviated as Log M, Log D, and Log A, respectively. All explanatory variables were log transformed.

2.3.1. Partitioning variation in cyanobacterial community

The type of ordination method (whether based on a linear model or that a unimodal model) appropriate for a particular cyanobacterial community across a gradient length was determined through detrended correspondence analysis (DCA) (Borcard et al., 1992). If the gradient length is smaller than 4, then a linear distribution (i.e., redundancy analysis [RDA]) is appropriate for cyanobacterial metrics. Meanwhile, canonical correspondence analysis (CCA) is appropriate for unimodal distributions. The RDA or CCA with variation partitioning was applied to assess the relative influence of environmental factors. The variation was partitioned into multiple components: a physical component (including WT, SD, and WS), a chemical component (including TN, TP, DIN, and DIP), a biological component (including the biomass of protozoa, rotifers, cladocerans, and copepods), and residual variation. A step-wise selection procedure (9999 permutations) was performed with adjusted R^2 (R^2_{adj}) as the selection criterion (Anderson and Cribble, 1998). Variance partitioning analysis was performed by the varpart function in the R package "vegan" and included a complete Venn diagram to visualize the result (Oksanen et al., 2013).

2.3.2. Generalized additive and liner mixed-effects models

In order to examine the seasonal cyanobacteria variability along water temperature gradient, a generalized additive model (GAM) approach was applied to identify potential thresholds via thin-plate regression splines. This technique enables the identification of different types of relationships between variables, and provides confidence intervals for the regression line that allow visual inspection of the significance of relationships (Salmaso et al., 2012). In this work, GAM models were applied using lake as a categorical variable, water temperature as a continuous explanatory variable,

$$\log(\text{Cyano}_{ij} + 1) = \alpha_j + S_j(\text{Temperature}_i) + \varepsilon_{ij}, \varepsilon_{ij} \sim N(0, \sigma^2) \quad (1)$$

where i and j are indices for the observations (monthly biomass of selected taxa and monthly water temperature) and the three lakes, respectively, and S_j is the smoothing function based on cubic regression spline. For each of the taxa, a graph is needed to visualize the function S_j . The "mgcv" package in R was used to optimise the amount of cubic spline smoothing. σ is the standard deviation of model error, ε , for GAM regression.

Multiple linear regressions with forward selection was used to explore the relationships between three bloom-forming taxa and environmental and biological factors. To account for the differences among regions within a lake (spatial heterogeneity), a linear mixed-effect model (LMM) with random intercepts for each region of each lake was used. Conditional R^2 , reflecting the goodness-of-fit of a model, was calculated (Nakagawa and Schielzeth, 2013).

2.3.3. Structural equation model

The biomass of different bloom-forming cyanobacteria in response to nutrients, temperature, and light availability and zooplankton

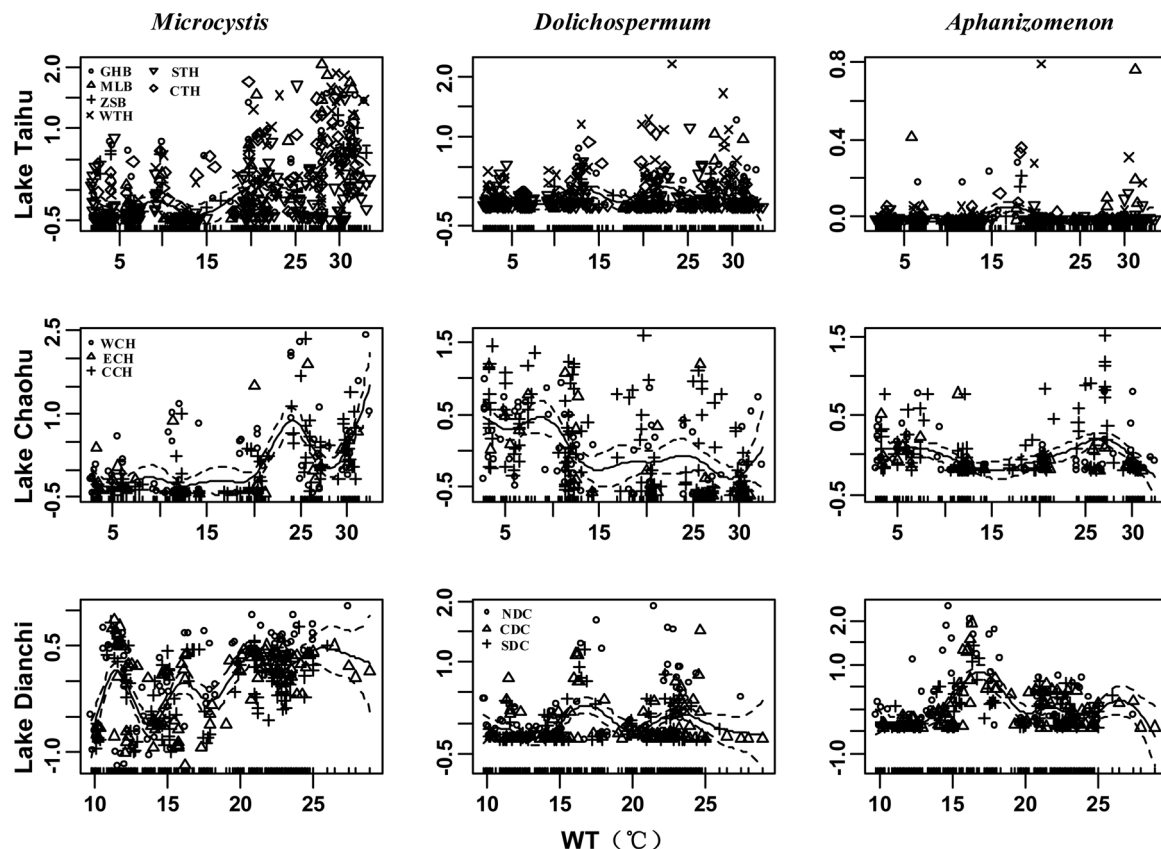


Fig. 6. Smoothing curves for three bloom-forming cyanobacteria along a water temperature (WT) gradient obtained by applying generalized additive model (GAM). The graphs from left to right show log cyanobacterial biomass for *Microcystis*, *Dolichospermum*, and *Aphanizomenon*, respectively. The black lines show the GAM regressions fitted to each relationship, with dotted lines giving the confidence interval of the mean trend line. Different shapes of points represent data from specific regions. Lake Taihu consists of Meiliang Bay (MLB), Zhushan Bay (ZSB), West Taihu (WTH), Center Taihu (MTH), Gonghu Bay (GHB), and South Taihu (STH). Lake Chaohu includes West Chaohu (WCH), Center Chaohu (CCH) and East Chaohu (ECH). Lake Dianchi, contains North Dianchi (NDC), Center Dianchi (CDC), and South Dianchi (SDC).

biomass was assessed using structure equation model (SEM). The a priori structural equation model describing the expected relationships among variables is illustrated in Fig. 2, and was used to test supposed causal structure between driving forces and species-specific biomass. SEM are often represented using path diagrams, where arrows indicate directional relationships between observed variables (Byrne, 2013). Composite variables (shown as circles), as typical latent variables without error variance, aggregate several predictor variables into a single factor that is often not directly measurable itself (Grace et al., 2010). In this study, the composite has the same scale as the first indicator by pre-multiplying "cause1" by 1. According to the results of multiple linear regressions, the links for both TP and DIN are simultaneously set to 1.0 as a composite score. A single set of composite scores explains the joint effect of multiple predictors on response variables. SEM is estimated using a maximum likelihood approach by the "lavaan" package in R (Rosseel, 2012). The goodness-of-fit of the SEM for each lakes can be evaluated using a chi-square (χ^2) test ($P > 0.05$ suggests there is no difference between the a priori model and the real relationships occurring in the data) (Hopcraft et al., 2012). Only the significant relationships ($P < 0.05$) amongst exogenous predictors are reported in a single causal network.

3. Results

3.1. Spatial-temporal variations of three cyanobacterial species in the three lakes

Non-diazotrophic *Microcystis*, as the most common bloom-forming

cyanobacterium, prevailed in the summer cyanobacteria in the studied lakes (Figs. 3 and 4). The biomass of *Microcystis* contributed approximately 88% and 79% to the total biomass of cyanobacteria in Lake Taihu and Lake Dianchi, respectively, whereas it was only 49% in Lake Chaohu. Regularly, *Microcystis* in Lake Taihu and Lake Chaohu began to achieve dominance in cyanobacterial communities during the increasing phase of water temperature in spring and summer, then its biomass decreased gradually during the decreasing phase of water temperature in autumn. By contrast, *Microcystis* dominance in Lake Dianchi could persist for up to 10 months and cover the majority of the lake's surface. The biomass of *Microcystis* (annual mean $\pm$ standard deviations) was as high as 41.8 ± 36.8 mg/L in Lake Dianchi, while this value was 22.4 ± 80.3 mg/L and 16.7 ± 33.7 mg/L in Lake Chaohu and Lake Taihu, respectively. Spatially, the distribution of *Microcystis* biomass showed a decreasing trend from the western to the eastern region of Lake Chaohu, and declining from the northern to the southern region of Lake Dianchi. Whereas, *Microcystis* biomass in the different regions of Lake Taihu varied in the order ZSB > MLB > WTH > CTH > GHB > STH.

Filamentous *Dolichospermum* prevailed in the winter and early spring cyanobacteria in some regions of Lake Chaohu and Lake Taihu. By contrast, *Dolichospermum* seldom gained dominance in Lake Dianchi across sampling periods, although it occupied a comparable biomass. Annual mean biomass of *Dolichospermum* was as high as 10.6 ± 18.3 mg/L in Lake Chaohu, while this value was 3.7 ± 10.2 and 0.9 ± 2.4 mg/L in Lake Dianchi and Lake Taihu, respectively. The biomass of *Dolichospermum* contributed 48% and 64% to the total cyanobacterial biomass in the middle and the eastern regions of Lake

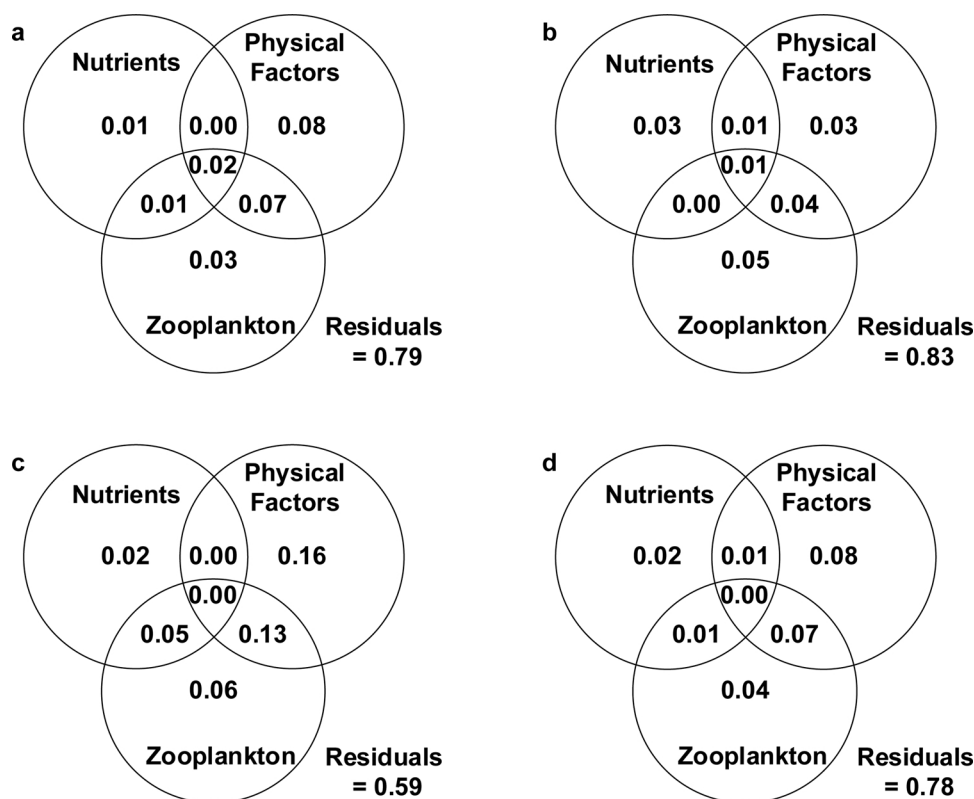


Fig. 7. Venn diagram representation of the partitioning of variation in cyanobacteria community matrices according to three sets of variables, nutrients, physical factors and zooplankton composition with different datasets: a) all three lakes, b) Lake Taihu, c) Lake Chaohu, d) Lake Dianchi. The value in the circle shows the adjusted unique contribution (R^2_{adj}) for each explanatory variables. Each area of overlap of the three circles is representative of the intersection in terms of their explained variation. The value of residuals represent the unexplained variation.

Chaohu, where it was higher than that of *Microcystis* from December to March of the next year. Furthermore, *Dolichospermum* in the western and the southern regions of Lake Taihu occupied relatively higher proportions at more than 25% and 13%.

Another filamentous genus, *Aphanizomenon*, prevailed in early spring cyanobacteria in the northern region of Lake Dianchi, where it occupied more than 92% to the total biomass of cyanobacteria in March 2009. The annual mean biomass of *Aphanizomenon* was 8.6 ± 30.5 mg/L in Lake Dianchi, and had an apparent decreasing trend from the northern to the southern regions. In comparison, *Aphanizomenon* biomass in Lake Chaohu reached a maximum value of 13.0 mg/L in July 2009, and contributed to 70% of total cyanobacteria, whereas it seldom achieve dominance in Lake Taihu.

3.2. Niche differentiation among the major bloom-forming species along environmental gradients

A proxy of algal biomass (Chl *a*) was positively correlated with WT, TP and TN across the three lakes (Fig. 5), while the total cyanobacteria biomass (CY) and the percentages of N_2 -fixing cyanobacteria (Nfix %) did not show a consistent pattern with WT, TP and TN, indicating lake-specific succession of genera. A weighted average was chosen to test environmental niche differentiation among three bloom-forming species (Table 1). The average water temperature weighted by *Microcystis* biomass (20.7 °C), was 2.7 °C and 4 °C higher than for *Aphanizomenon* biomass and *Dolichospermum* biomass, respectively. The difference in weighted average water temperature between *Microcystis* and two filamentous genera was even bigger in Lake Chaohu and Lake Taihu than it was in Lake Dianchi. Therefore, with the exception of Lake Dianchi, Nfix % had a strong negative correlation with WT.

Furthermore, we found important differences in the weighted average nutrients (TN and TP) between *Microcystis* and two filamentous genera. For the subset of Lake Chaohu, the difference was so large that Nfix% was negatively related to TN and TP. Unexpectedly, Nfix% had a strong positive relationship with TN:TP, albeit negatively significant

with DIN. In comparison, the occurrences of high Nfix% were associated with lower TN:TP in some regions of Lake Taihu and Lake Dianchi (Table 2).

With a few exceptions, the GAM results revealed that multi-humped distributions appeared between water temperature and species-specific biomass (Fig. 6). The critical water temperature above which non-diazotrophic *Microcystis* gradually became the dominant genus was ~ 20 °C in Lake Taihu and Lake Chaohu, in contrast to a long-lasting dominance in Lake Dianchi. Interestingly, troughs of *Microcystis* biomass in Lake Dianchi corresponded to peaks of two filamentous cyanobacteria at ~ 17 °C. Similarly, the temperature-dependence of *Dolichospermum* in Lake Chaohu was relatively wide, but the highest biomass mainly occurred at the temperature range of 5 $\sim$ 15 °C. Therefore, despite a considerable overlap, niche differences between *Dolichospermum* and *Microcystis* or *Aphanizomenon* were mostly significant in Lake Chaohu.

3.3. Partitioning the impact of environmental and biological factors on cyanobacteria

To evaluate which environmental variables influenced cyanobacterial composition the most, the variations in cyanobacterial communities composition explained by nutrient, physical, and zooplankton variables and their overlap were presented in Fig. 7. Physical factors were the most important drivers across the three lakes, with explained variations of 3%–16%. Zooplankton community was identified as the secondary driver and its effectiveness depended on their interactions with physical factors. In comparison, nutrients played a less role in manipulating cyanobacterial communities with only 1%–3% of the variation explained. Together, the R^2_{adj} value for cyanobacterial community's matrices in Lake Chaohu was up to 41%, compared with Lake Taihu ($R^2_{adj} = 17\%$) and Lake Dianchi ($R^2_{adj} = 23\%$).

The LMM analysis revealed *Microcystis* and two filamentous taxa had different tolerances and responses to the biomass of the zooplankton community. From Table 3, results showed that the biomass of metazoan zooplankton, including either cladocerans or copepods, was

Table 3
Linear mixed-effects model explaining the biomass of three bloom-forming cyanobacteria based on lake-specific and across-lake datasets. For the models, random effects for each region were evaluated. All regression models were significant ($P < 0.001$), and the standard deviation of the random effects and residuals are given (σ). Conditional R^2 is interpreted as variance explained by both fixed and random factors.

Lake	Dependent variable	Linear model	σ -intercept	σ -random	Conditional R^2	df	BIC
Taihu	Log M	$0.50^{***} \text{LogWT} + 0.27^{***} \text{LogTP} + 0.17^{***} \text{LogTN} - 0.20^{***} \text{LogSD} + 1.15^{***} \text{LogPro} + 0.43^{***} \text{LogRot} + 0.54^{***} \text{LogClad}$	0.08	0.45	0.43	477	686
	Log D	$0.33^{***} + 0.21^{***} \text{LogWT} + 0.14^{***} \text{LogTN} + 0.08^{***} \text{LogDIP} - 0.23^{***} \text{LogSD} + 0.69^{***} \text{LogPro}$	0.05	0.27	0.21	472	193
	Log A	$0.02^{***} \text{LogWT} + 0.09^{***} \text{LogRot} - 0.03^{***} \text{LogCop}$	0.00	0.06	0.08	499	-1267
Chaohu	Log M	$1.00^{***} \text{LogWT} + 0.75^{***} \text{LogTP} - 0.32^{***} \text{LogSD} + 1.59^{***} \text{LogPro} + 0.54^{***} \text{LogRot}$	0.06	0.33	0.68	241	215
	Log D	$1.21^{***} + 0.42^{***} \text{LogTP} - 0.49^{***} \text{LogDIN} - 0.42^{***} \text{LogClad} - 0.40^{***} \text{LogCop}$	0.00	0.50	0.23	252	412
	Log A	$0.07^{***} - 0.13^{***} \text{LogDIN} + 0.26^{***} \text{LogCop}$	0.00	0.29	0.07	252	126
Dianchi	Log M	$-1.54^{***} + 2.04^{***} \text{LogWT} + 0.50^{***} \text{LogTP} - 0.29^{***} \text{LogDIN} + 0.40^{***} \text{LogPro} - 0.28^{***} \text{LogRot} + 0.31^{***} \text{LogCop}$	0.00	0.36	0.54	303	315
	Log D	$-0.76^{***} + 0.56^{***} \text{LogTP} + 0.44^{***} \text{LogTN} - 0.20^{***} \text{LogDIP} + 0.57^{***} \text{LogSD} - 0.13^{***} \text{LogClad}$	0.00	0.34	0.22	278	251
	Log A	$1.35^{***} - 1.41^{***} \text{WT} + 0.59^{***} \text{LogTP} - 0.15^{***} \text{LogDIP} + 0.58^{***} \text{LogSD} + 0.42^{***} \text{LogCop}$	0.00	0.44	0.26	278	387
All lakes	Log M	$0.85^{***} \text{LogWT} + 0.62^{***} \text{LogTP} - 0.21^{***} \text{LogDIN} - 0.12^{***} \text{LogDIP} - 0.23^{***} \text{LogSD} + 0.88^{***} \text{LogPro} + 0.40^{***} \text{LogRot} + 0.29^{***} \text{LogClad}$	0.11	0.42	0.61	990	1223
	Log D	$0.22^{***} + 0.21^{***} \text{LogWT} + 0.13^{***} \text{LogTP} - 0.11^{***} \text{LogDIN} + 0.61^{***} \text{LogPro} - 0.28^{***} \text{LogRot} - 0.16^{***} \text{LogClad}$	0.19	0.40	0.23	1054	1208
	Log A	$0.13^{***} \text{LogTP} + 0.16^{***} \text{LogSD} - 0.25^{***} \text{LogRot} - 0.14^{***} \text{LogClad} + 0.27^{***} \text{LogCop}$	0.24	0.31	0.41	1021	706

Note: *Microcystis* (Log M in mg L^{-1}), *Dolichospermum* (Log D in mg L^{-1}), *Aphanizomenon* (Log A in mg L^{-1}), water temperature (WT in $^{\circ}\text{C}$), total nitrogen (TN in mg L^{-1}), total phosphorus (TP in mg L^{-1}), dissolved inorganic nitrogen (DIN in mg L^{-1}), dissolved inorganic phosphorus (DIP in mg L^{-1}), secchi depth (SD in cm), protozoans (Pro in mg L^{-1}), rotifers (Rot in mg L^{-1}), cladocerans (Clad in mg L^{-1}) and copepods (Cop in mg L^{-1}).

The significance of the regression coefficients is indicated by:

- *** $P < 0.001$.
- ** $0.001 < P < 0.01$; and
- * $0.01 < P < 0.05$.

positively related to *Microcystis* biomass, but negatively related to *Dolichospermum* biomass. Besides, *Microcystis* biomass was positively correlated with rotifer biomass in Lake Taihu and Lake Chaohu, and with protozoa biomass in all three lakes.

3.4. SEM predicting biomass of three bloom-forming cyanobacteria

The predictor variables of the SEM were aggregated into the composites, in order to determine the importance of environmental drivers. The chi-square associated with SEM describing the biomass of the three taxa suggested the dataset in Lake Taihu supported a priori interpretation ($\chi^2 = 8.33$, d.f. = 4, $P = 0.080$) (Fig. 8b). The SEM explained 31% of the variation in *Microcystis* biomass, in contrary to a little of variation in *Dolichospermum* biomass (13%) and *Aphanizomenon* biomass (4%). For the subset of Lake Taihu, water temperature was the most significant factor in explaining the variations of *Microcystis* biomass (standardized path = 0.31), followed by phosphorous concentration and zooplankton biomass with standardized path values of 0.21 and 0.28, respectively. *Dolichospermum* biomass was also positively related to water temperature (0.26) and negatively related to Secchi depth (-0.22). Nitrogen concentrations with respect to TN and DIN were not correlated with the biomass of the two filamentous taxa and *Microcystis*.

For the subset of Lake Chaohu, the dataset on the biomass of the three genera supported the SEM model ($\chi^2 = 4.61$, d.f. = 4, $P = 0.33$) (Fig. 8c). The SEM described 70% of the variation in *Dolichospermum* biomass, which was negatively related to nitrogen concentration (standardized path = -0.29), water temperature (-0.27), and zooplankton biomass (-0.24). The SEM explained 50% of variation in *Microcystis* biomass, which was positively associated with water temperature and phosphorus concentration. In comparison, the SEM accounted for only 9% of the variation in *Aphanizomenon* biomass, but its correlations with environmental factors were similar with that of *Dolichospermum* biomass.

The SEM described more than 50% of variation in *Microcystis* biomass and was supported by the dataset in Lake Dianchi ($\chi^2 = 9.32$, d.f. = 4, $P = 0.054$) (Fig. 8d). Water temperature had a predominantly positive effect on *Microcystis* biomass (standardized path = 0.49), and the next most important correlates were phosphorus concentration (0.23) and zooplankton biomass (0.21). The SEM accounted for 20% and 16% of the variation in biomass of *Dolichospermum* and *Aphanizomenon*, respectively. The two filamentous taxa were positively associated with the phosphorus concentration and Secchi depth, whereas nitrogen concentration had a positive relationship with *Aphanizomenon* biomass.

4. Discussion

In the three lakes studied, our application of statistical models showed that cyanobacterial communities appear to be driven strongly by physical factors, which was in agreement with previous findings from other freshwater ecosystems (Steinberg and Hartmann, 1988; Soares et al., 2009). Notably, the seasonal shifts in bloom-forming species may be largely attributed to their relative temperature adaptation, with warmer temperature favoring non-diazotrophic *Microcystis* over the filamentous *Dolichospermum* and *Aphanizomenon* (Takano and Hino, 1998; Paerl and Otten, 2015). Nevertheless, the GAM revealed that the two filamentous species had comparable biomass even at relatively high temperature, providing potential support to co-dominance patterns. This finding is consistently supported by several lines of evidence. (i) High correlation coefficient values among response variables in the SEM (i.e. variables on the far right of Fig. 8) provided insights into associations among bloom-forming species. For instance, significant correlations were observed between *Microcystis* and *Dolichospermum* in Taihu, as well as *Dolichospermum* and *Aphanizomenon* in Lake Chaohu. (ii) The difference amongst taxa in weighted average water

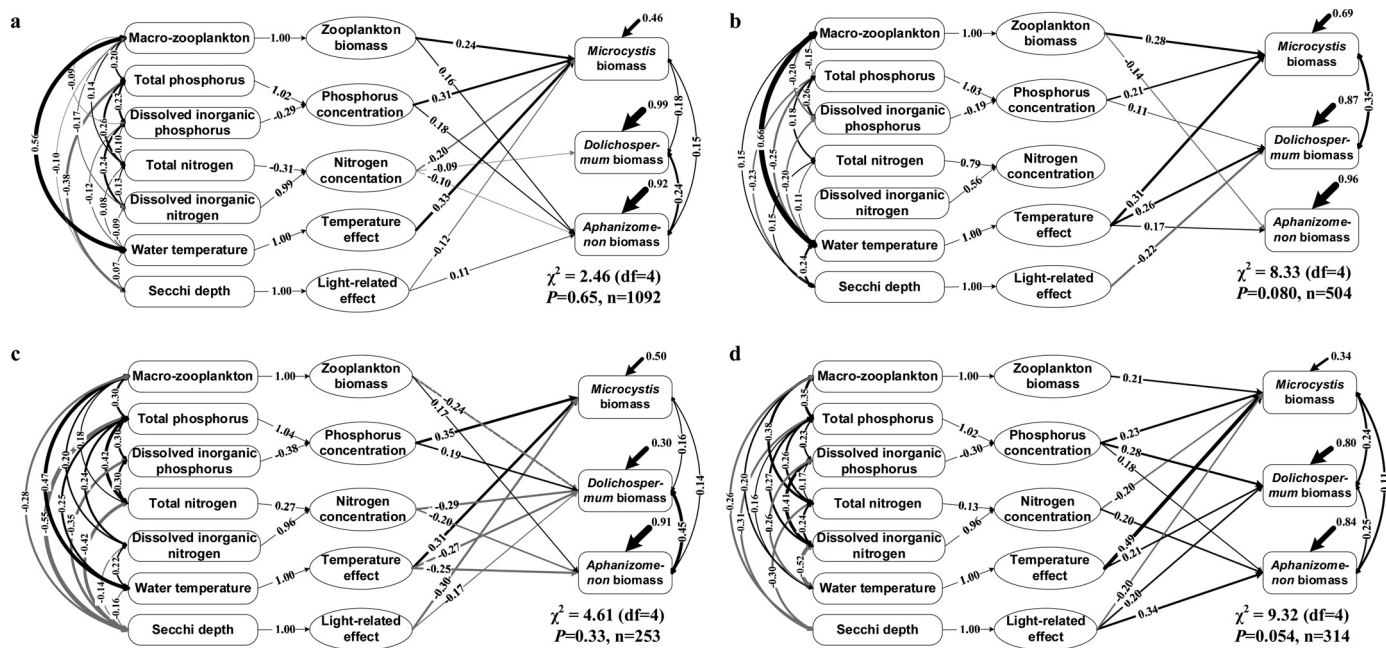


Fig. 8. The result of structure equation model assessing three bloom-forming cyanobacteria in different dataset: a) all three lakes, b) Lake Taihu, c) Lake Chaohu, d) Lake Dianchi. Solid lines indicate significant paths ($P < 0.05$) and are weighted according to their standardized path strength. Black lines are positive path strengths, while grey lines mean negative path strengths. Without linear link between predictors and response variables represent non-significant paths. Curved double-headed arrows represent the correlation between the exogenous or endogenous variables.

temperature confirmed niche differentiation between *Microcystis* and two filamentous taxa in Lake Taihu and Lake Chaohu (Table 1). In comparison, competitive relationships among three species in Lake Dianchi tended to be moderate and neutral, because of mild weather for the entire year (Fig. 4). The results echoes the hypothesis in experimental microbial communities that temperature fluctuation facilitates coexistence of competing species (Jiang and Morin, 2007).

Apart from direct effects, warming temperature interacts with nutrients loading and underwater light conditions to favor non- N_2 -fixing *Microcystis*. In the three polymictic lakes, climate warming may increase nutrient concentrations by enhancing mineralization and anoxia-mediated sediment P release (Kosten et al., 2012). In addition, *Microcystis* which occurs as colonies of different sizes, can regulate its buoyancy for the optimum utilization of nutrients and light resources; this may be responsible for the release of sediment-bound P and increase soluble reactive P in and near sediments (Brunberg, 1995; Head et al., 1999). Therefore, outcomes of the SEM underlined that the competitive advantage of *Microcystis* can more likely be attributed to synergistic effects between water temperature and P concentrations across the three lakes. Congruent with biomass accumulation, light limitation may be crucial for *Microcystis* to outcompete the two filamentous taxa, related to more efficient use of light for supporting photosynthetic growth and nutrient sequestration than diazotrophic genera (Paerl et al., 2014). This evidence points to a positive feedback because Secchi depth decrease was often a consequence of high biomass of *Microcystis* (Table 3). The results of SEM in Lake Dianchi revealed the biomass of *Aphanizomenon* or *Dolichospermum* increased with increasing water transparency (Fig. 8d). This observation partly explains why distinct troughs of *Microcystis* biomass corresponded to crests of two filamentous taxa in early spring (Fig. 5).

Another potential driver of the competitive balance, and especially the responses of two filamentous taxa, may be the existing sources of N and P stored and cycled in each study lake. In Lake Taihu, there is a possible role of regenerated nutrients in explaining the dominance of *Microcystis*, because it effectively competes for regenerated N from sediments and the water column (McCarthy et al., 2007). Controlled experiments with a series of large (1000 L) in-lake mesocosms during

summer (Paerl et al., 2014), demonstrated *Microcystis* and other non- N_2 fixing genera can maintain dominance by outcompeting N_2 -fixing taxa for the existing sources of N and P in this lake. In Lake Dianchi, there was a counterintuitive relationship between N concentrations and diazotroph biomass (Fig. 8d and Table 3), since N_2 fixers are expected to dominate when N concentrations are lower (Schindler, 2012). The answer to this phenomenon likely pertains to extremely high nutrient cycling in ecosystem of Dianchi Lake (Shan et al., 2014). *Microcystis* blooms contribute large amounts of “new” N and P as fuel for hypoxia and anoxia, because *Microcystis* colony regularly close associations with heterotrophic bacteria present around colonies to enhance ‘phycosphere’ – scale nutrient recycling (Cottingham et al., 2015). In the Lake Chaohu, *Dolichospermum* achieved dominance in the season when nitrogen concentrations in the ambient water were relatively high, which would buttress the field observations in four eutrophic lakes in South Central Wisconsin, USA (Miller et al., 2013). Because diazotrophic taxa with a capacity for nitrogen fixation will have higher cellular N:P ratios than non-diazotrophic taxa (Klausmeier et al., 2004), TN:TP ratios in the different regions of Chaohu varied in the sequence of $ECH > CCH > WCH$.

Biological interaction affecting cyanobacteria succession come from the differences in species-specific defense strategies against grazing pressures. The LMM analysis indicated that the biomass of cladocerans and copepods, were positively related to *Microcystis* biomass across the three study lakes. Filamentous *Dolichospermum* biomass, by contrast, was negatively related to the biomass of metazoan zooplankton (Table 3). Grazing theory suggests large-bodied *Daphnia* species and calanoid copepods can manipulate algal size structure (Elser, 1999; Marino et al., 2002), and some copepods can tolerate the ingestion of filamentous cyanobacteria (Kâ et al., 2012; Panosso et al., 2003). Grazing by copepods may shorten the filament length of less toxic strains, making them palatable to other smaller cladocerans (Chan et al., 2004). In addition, the value of correlations between macro-zooplankton biomass and water temperature in the SEM exceeded the reference level for 0.35 (Grace and Bollen, 2008). There is evidence suggesting that the interaction between temperature and zooplankton is the next most important factor in influencing succession. Considering a

relatively large biomass of metazoan zooplankton (annual mean value, 571 in./L in Lake Dianchi, 415 in./L in Lake Chaohu, 231 in./L in Lake Taihu), it seems reasonable to infer that high temperature increases the frequency, duration, and intensity of *Microcystis* blooms, selecting for zooplankton adapted to coexist with, rather than consume, the *Microcystis* (Ger et al., 2014; Perga et al., 2013; Wilson et al., 2006).

The greatest challenge of correlational field data is the difficulty of inferring causality and predicting trends. In the present study, the SEM was developed by data-driven means, not just to cope with uncertainty within field observations, but to provide a means to link different variables to general theoretical concepts. Generally, the SEM is more sensitive to changes in its input variables, but this method is useful when sufficient and reliable monitoring data covering large spatio-temporal scales is available (Beaulieu et al., 2013; Otten et al., 2012). This study has limitations, in that within-species strain variability affects cyanobacteria population responses to environmental conditions (Xiao et al., 2017). For instance, toxic *Microcystis* strains can suppress the growth of the two filamentous taxa, owing to a wide range of allelochemicals, particularly microcystins (Berry et al., 2008; Ma et al., 2015; Zhang et al., 2016). A few investigations to date have attempted to link the dynamics of *Microcystis* genotypes and morphotypes with environmental conditions (Davis et al., 2009; Wu et al., 2014). Overall, we predict that the advantage of *Microcystis* over two filamentous species will be reinforced in future, because toxic *Microcystis* dominance increases steeply with climate warming and eutrophication.

5. Conclusion

Many studies have been conducted on the drivers of cyanobacteria blooms in the past decades, but knowledge of in situ succession patterns of bloom-forming species and their response to environmental conditions and trophic change remains inadequate. In this study, the combined effects of temperature, nutrients, and zooplankton on the spatio-temporal variations of cyanobacteria were assessed by statistical models. The SEM accounted for the linkage among different variables using general theoretical concepts. The results of SEM showed that *Microcystis* biomass was influenced primarily by water temperature and P concentrations. By contrast, the biomass of the two filamentous N_2 -fixing taxa exhibited lake-specific responses. Considering future scenarios of warming and N:P stoichiometry imbalance, we suggest that reducing P may be feasible for long-term control of non-diazotrophic *Microcystis* until sediment nutrient storage is exhausted. Decision-makers require comparative analysis of results from similar bloom-dominated lakes when the data for the lake of interest is insufficient.

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